Journal of Power Sources 268 (2014) 14–18

Contents lists available at ScienceDirect

Journal of Power Sources

journal homepage: www.elsevier.com/locate/jpowsour

Short communication

A phosphorus-free anolyte to enhance coulombic efficiency of microbial fuel cells

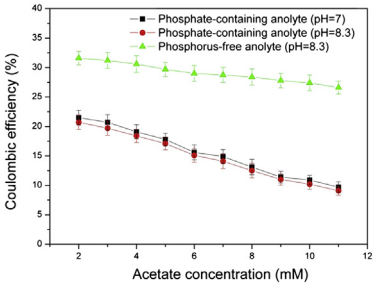
Xinhua Tang [a, b], Haoran Li [c], Zhuwei Du [c], How Yong Ng [a, *]

[a] National University of Singapore, Department of Civil and Environmental Engineering, Centre for Water Research, Singapore 117576, Singapore
[b] National University of Singapore, NUS Graduate School for Integrative Sciences and Engineering, Singapore 117456, Singapore
[c] Chinese Academy of Sciences, Institute of Process Engineering, National Key Laboratory of Biochemical Engineering, Beijing 100190, China

HIGHLIGHTS

- The phosphorus-free anolyte inhibits the growth and reproduction of suspended cells.
- The phosphorus-free anolyte greatly reduces the suspended cell mass.
- The phosphorus-free anolyte enhances the coulombic efficiency of MFCs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form
3 June 2014
Accepted 3 June 2014
Available online 11 June 2014

Keywords:
Coulombic efficiency
Phosphorus-free anolyte
Suspended biomass
Microbial fuel cells

ABSTRACT

In this study, a phosphorus-free anolyte is prepared by using bicarbonate to replace phosphate buffer for application in two chamber microbial fuel cells (MFCs). Optical density test and Bradford protein assay shows that this phosphorus-free anolyte effectively inhibits the growth and reproduction of microorganisms suspended in the solution and greatly reduces the suspended cell mass. As a result, it considerably enhances the coulombic efficiency (CE) of MFCs. When the acetate concentration is 11 mM, the CE of the MFC using the pH 7 phosphate-containing anolyte is 9.7% and the CE with the pH 8.3 phosphate-containing anolyte is 9.1%, while the CE of the MFC using the phosphorus-free anolyte (pH 8.3) achieves 26.6%. This study demonstrates that this phosphorus-free anolyte holds the potential to enhance the feasibility for practical applications of MFCs.

1. Introduction

Microbial fuel cell (MFC) is a bioelectrochemical system that converts chemical energy in organic matters into electrical energy by catalysis of microorganisms [1–3]. As a novel biotechnology for wastewater treatment, renewable energy production, biosensor, bioremediation and chemical synthesis, MFC has drawn much attention and has achieved great progress in the past decade [4–8]. Currently, one of the main challenges for improving MFC applications is to increase the electron recovery from the substrates (coulombic efficiency, CE) as the CE of MFCs is still too low [9–13]. The CE, defined as the ratio of the amount of electrons transferred through the circuit to the amount of electrons theoretically delivered by the substrate, is one of the most important criteria for MFC performance evaluation. The CE is mainly diminished by growth and reproduction of microorganisms, competitive processes such as fermentation, and aerobic respiration due to the diffusing of oxygen into the anode chamber [10,14]. Therefore, it is of great significance to address these challenges to enhance CE in order to create efficient MFCs for these applications.

* Corresponding author. Tel.: +65 65164777; fax: +65 67744202.
E-mail address: howyongng@nus.edu.sg (H.Y. Ng).

http://dx.doi.org/10.1016/j.jpowsour.2014.06.009
0378-7753/

Coulombic efficiency typically depends on the electrolyte composition, inocula, reactor design and operation [15]. Previous studies demonstrates that biofilm formed on the electrode surface is primarily responsible for the electricity production and microorganisms suspended in the anolyte contributes nothing or only a little to the electricity production in MFCs [16–19]. Microorganisms suspended in the anolyte, however, compete with microorganisms attached on anode surface to utilize organics for fermentation and aerobic respiration, which consequently lowers the CE of MFCs. As a result, inhibiting the growth and reproduction of microorganisms suspended in the solution can be an effective method to enhance the CE of MFCs. On the other hand, phosphate buffer is popularly added into anolyte in MFC studies to help stabilize the solution pH for exoelectrogens and to enhance power production [20–22]. Adding phosphate into MFCs, however, not only leads to the eutrophication of water bodies if the effluents are discharged without effective removal of phosphates, but also contributes to the growth and reproduction of suspended microorganisms as phosphorus is an essential element for microorganism to synthesize ATP, phospholipids, DNA and RNA. Therefore, the strategy here is to prepare a phosphorus-free anolyte by using bicarbonate as an alternative of phosphate buffer to inhibit the growth and reproduction of suspended microorganisms. This study demonstrates that this phosphorus-free anolyte greatly reduces the biomass suspended in the solution and considerably enhances CE of MFCs.

2. Materials and methods

2.1. Two-chamber MFC construction and operation

The MFCs were constructed by joining two bottles (height 22 cm, diameter 3.8 cm) with the proton exchange membranes (Nafion 117, Dupont) held by a clamp in the tube (inner diameter 2 cm) separating the two chambers, as previously described [23]. The proton exchange membranes were sequentially boiled in H_2O_2 (30%), deionized water, 0.5 M H_2SO_4, and deionized water. The anode and cathode were graphite felts (3 cm × 0.6 cm × 8 cm) placed in each chamber with an electrode spacing of 7 cm. Graphite felts were pretreated by immersing successively in 1 M NaOH for 12 h and then 1 M H_2SO_4 for 12 h, followed by rinsing with DI water. Cathode were prepared by coating Pt/C catalysts (0.5 mg cm^{-2} Pt) onto the graphite felt surface using 5% Nafion solutions as the binder [24]. In brief, Pt/C catalyst was firstly mixed and dispersed well in the Nafion and ethanol solution by ultrasonication. Then, the dispersion was coated onto the graphite felt followed by drying at room temperature. Titanium wire across an external resistance of 500 Ω was used to connect the anode and cathode.

The MFCs was inoculated using a mixed bacterial culture from another MFC in the laboratory which had been running for over two years [25]. Phosphate-containing anolyte was composed of 50 mM phosphate buffer solution (pH 7 and pH 8.3), NH_4Cl (0.31 g L^{-1}), KCl (0.13 g L^{-1}), NaCl (2.9 g L^{-1}), metal salt (12.5 mL L^{-1}) and vitamin (5 mL L^{-1}) solutions [26]. Phosphorus-free anolyte was the same as phosphate-containing anolyte except that 50 mM bicarbonate (pH 8.3) was used to replace the phosphate buffer. The catholyte was composed of NH_4Cl (0.31 g L^{-1}), KCl (0.13 g L^{-1}), NaCl (2.9 g L^{-1}) and 50 mM Tris–HCl buffer.

The anode chamber was maintained under anaerobic condition, while the cathode chamber was purged with sterile air (40 mL min^{-1}). The MFCs were operated in a temperature-controlled incubator at 30 °C in a fed batch mode. Phosphate-containing anolyte (pH 7 and pH 8.3) and phosphorus-free anolyte with acetate were used respectively in three MFCs. Anolyte with different acetate concentration (2 mM–11 mM) was replaced at the end of each batch when the voltage dropped below 20 mV. The feeding solution had been sparged with high purity nitrogen gas for 30 min to remove the oxygen before adding into the anode chamber. All the experiments were carried out in duplicate and the average values with standard deviation were obtained.

2.2. Analyses

Cell voltage (V) across the external resistance (R) was measured and recorded using a data acquisition system (AD8201H, Ribohua Co., Ltd) and current (I) was calculated by $I = V/R$. Charge passing through the external circuit (Coulombs, C_p) was obtained by integrating the current over time. The theoretical amount of charge (C_t) that could be produced from acetate consumed was calculated by $C_t = Fbcv$, where F was the Faraday's constant (96,500C mol^{-1} of electrons), b was the number of mole of electrons produced per mole of acetate ($b = 8$ for acetate), and c was the consumed substrate concentration and v was the anolyte volume (200 mL). CE was obtained by the equation $C_e = C_p/C_t$.

Acetate concentrations were analyzed using a gas chromatograph (Agilent, 6890) as previously described [27]. The pH of the anolyte was measured by a pH meter (PHS-2F, INESA) and the conductivity of the anolyte was measured by a conductivity meter (S700, Mettler Toledo). Microorganisms formed on graphite felt surface were examined by environmental scanning electron microscopy (SEM) (Quanta 200, FEI). Suspended cell mass was evaluated by two methods at the end of each batch: the turbidimetric method using a spectrophotometer to measure the optical density at 600 nm (OD_{600}) [28], and Bradford protein assay to measure the absorbance at 595 nm.

3. Results and discussion

3.1. Electricity generation from MFCs

When MFCs become stable in power generation, approximately 220 mV across an external resistance of 500 Ω is produced in the three MFCs (Fig. 1). SEM images clearly show that microorganisms adhere on graphite felt anode surface to form biofilm, which is expected to generate electricity in MFCs. In order to confirm whether the biofilm is responsible for the electricity generation in these MFCs, the anolyte in the stably running MFCs is removed and replaced. After the replacement, voltage generation rapidly rises to

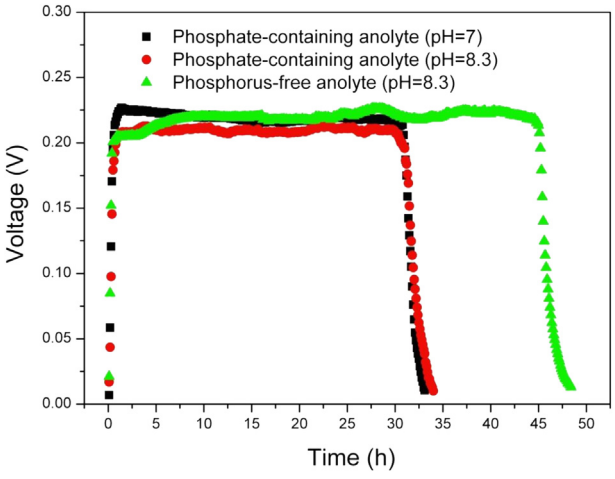


Fig. 1. Voltage generation from MFCs with the phosphorus-free anolyte and the phosphate-containing anolyte (acetate concentration: 2 mM).

a maximum (less than 30 min) and stabilizes at levels to those observed prior to anolyte replacement. The result that replacement of the anolyte surrounding the anode without changing the voltage output capacity confirms that microorganisms attached on the anode surface are responsible for electricity production in these MFCs, which is consistent with the previous reports [17–19]. This result also demonstrates that microorganisms suspended in the anolyte do not contribute to the power production in theses MFCs. These suspended microorganisms, however, compete with the microorganisms attached on anode surface to consume organics for fermentation and aerobic respiration, which accordingly decreases the CE of MFCs. As a result, inhibiting the growth and reproduction of microorganisms suspended in the solution can be an effective method to enhance the CE of these MFCs.

In each batch cycle, voltage generation from MFCs rapidly rises to a maximum and sustains at a certain level (200–230 mV) (Fig. 1). When acetate is limited, the voltage output begins to decline and drops gradually to 20 mV. The stable voltage output is almost the same in the three MFCs except that voltage output with the phosphorus-free anolyte sustains much longer than that with the phosphate-containing anolyte. A longer voltage generation time implies a higher CE. Also, this result indicates that biofilm on anode surface in all the anolytes is stable and consistently produces electricity, even maintained in anolyte without phosphorus.

3.2. The pH and conductivity

The pH of the anolyte affects the performance of MFCs. In the absence of buffer, a broad pH change can result in much lower current production [20]. Therefore, buffer is commonly used in MFCs to maintain a suitable pH for exoelectrogens. The pK_2 of the phosphate buffer is 7.2 and the pK_1 of the phosphorus-free buffer is 6.4. Consequently, both of them have strong buffering capacity in the pH range of 6–8.5. The pH changes very slightly (0.01–0.03 unit) when 10 mM acetate is added into the three anolyte (Table 1). After one batch cycle for power production with 10 mM acetate, the pH drops only 0.24, 0.26 and 0.27 unit in the pH 7 phosphate buffer anolyte, the pH 8.3 phosphate buffer anolyte and the phosphorus-free anolyte, respectively. These results indicate that all the three buffers have strong capacity in stabilize the solution pH.

In MFCs, a relatively higher conductivity is considered beneficial to power generation and CE because it facilitates proton transfer in the solution [29]. In this study, the conductivity of the phosphorus-free anolyte is 9.25 mS cm^{-1}, smaller than the 10.76 mS cm^{-1} of the pH 7 phosphate buffer anolyte and the 11.87 mS cm^{-1} of the pH 8.3 phosphate buffer anolyte (Table 1). Therefore, the relatively smaller conductivity of the phosphorus-free anolyte is not favorable for the CE of MFCs.

3.3. Suspended microorganisms and CE

Suspended cell mass is popularly evaluated by measuring the optical density at 600 nm (OD_{600}) as the optical density of a cell suspension is proportional to its biomass concentration [30,31]. The OD_{600} of the suspended microorganisms in MFCs fed with different acetate concentration is measured at the end of each batch cycle as shown in Fig. 2. When acetate concentration increases from 2 mM to 11 mM, the OD_{600} boosts sharply from 0.136 to 0.389 in the pH 7 phosphate buffer anolyte and increases from 0.126 to 0.384 in the pH 8.3 phosphate buffer solution. Larger suspended cell mass is detected in a higher acetate concentration which has a longer batch cycle time. This finding demonstrates that suspended cell mass in MFCs increases significantly due to the growth and reproduction of suspended microorganisms. The OD_{600} in the phosphorus-free anolyte, however, is quite stable; when acetate concentration increases from 2 mM to 11 mM, the OD_{600} only increases slightly from 0.042 to 0.091, much smaller than the corresponding values in the phosphate-containing anolyte.

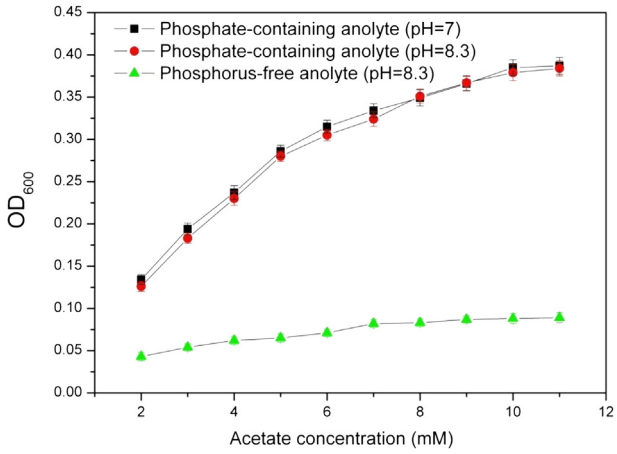


Fig. 2. The optical density (OD_{600}) of the suspended microorganisms in MFCs fed with different acetate concentration.

Bradford protein assay is employed to measure the suspended biomass more accurately. The protein concentration increases from 0.072 mg mL^{-1} to 0.294 mg mL^{-1} in the pH 7 phosphate buffer anolyte and increases from 0.066 mg mL^{-1} to 0.290 mg mL^{-1} in the pH 8.3 phosphate buffer anolyte, when acetate concentration changes from 2 mM to 11 mM (Fig. 3). The corresponding protein concentration in the phosphorus-free anolyte, however, only increases slightly from 0.012 mg mL^{-1} to 0.038 mg mL^{-1}.

These results demonstrated that, compared with the phosphate-containing anolyte, the phosphorus-free anolyte can effectively inhibit the growth and reproduction of suspended microorganisms in MFCs. As a result, this phosphorus-free anolyte greatly reduces the biomass suspended in the solution.

The CE of MFCs fed with different acetate concentration is shown in Fig. 4. The CE of the MFC using the pH 7 phosphate buffer anolyte declines remarkably from 21.5% to 9.7% and the CE with the pH 8.3 phosphate buffer anolyte declines from 20.7 to 9.1%, when the acetate concentration increases from 2 mM to 11 mM. The corresponding CE of the MFC using the phosphorus-free anolyte only decreases from 31.6% to 26.6%. These results show that the phosphorus-free anolyte considerably enhances the CE of the MFC compared with the phosphate-containing anolyte.

The much higher CE using the phosphorus-free anolyte is due to the significantly reduced microorganisms suspended in the anolyte

Table 1
The pH and conductivity of different anolyte.

Anolyte	pH	pH (+acetate 10 mM)	Conductivity (mS cm^{-1})	Conductivity (+acetate 10 mM) (mS cm^{-1})
pH 7 phosphate-containing anolyte	7.01	7.02	10.76	11.29
pH 8.3 phosphate-containing anolyte	8.31	8.33	11.87	12.38
Phosphorus-free anolyte	8.30	8.33	9.25	9.79

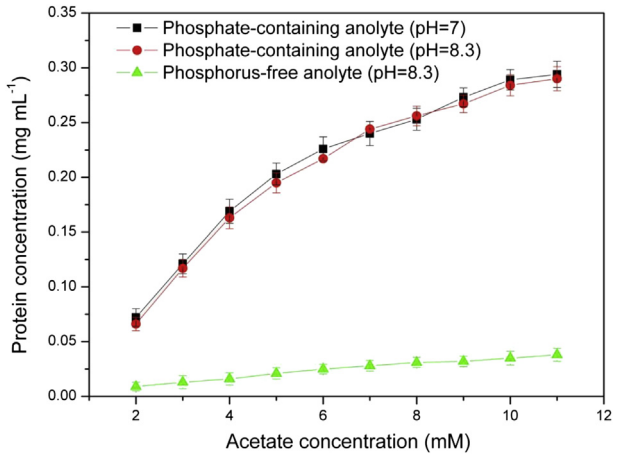


Fig. 3. The protein concentration of the suspended microorganisms in MFCs fed with different acetate concentration.

which are not responsible for the electricity generation in the MFCs. The reduction of cell mass suspended in the solution implies that less substrate is consumed by suspended microorganisms and more substrate is left for microorganisms attached on anode surface for electricity generation.

The phosphorus-free anolyte can effectively inhibit the growth and reproduction of suspended microorganisms. Nevertheless, it does not affect the biofilm formed on anode surface for power generation. The biofilm formed on anode surface maintained in the phosphorus-free anolyte is very stable and generate electricity with little deterioration in performance during the entire test period. The explanation could be that microorganisms attached on the anode surface could take advantage of the phosphorus sources from the dead cells within the biofilm.

An enhanced CE can undoubtedly improve the practical applications of MFCs. Traditional aerobic wastewater treatment such as activated sludge is energy intensive and costly; in the U.S., about 3% of electricity produced goes to wastewater treatment and the annual cost for wastewater treatment is as high as $25 billion [5]. Compared with aerobic treatment, the greatest advantages of MFC technology is that energy can be recovered from organics in wastewater. The power density and CE are typically much smaller when there is no buffer in the solution, while adding phosphate buffer into MFCs can lead to the eutrophication problem [20].

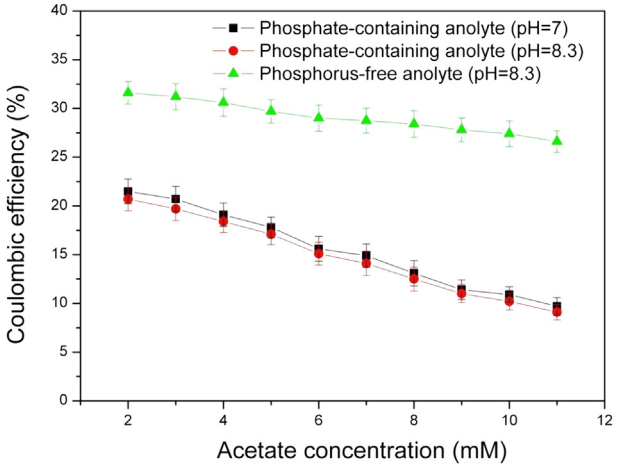


Fig. 4. Coulombic efficiency of MFCs fed with different acetate concentration.

Consequently, MFC with bicarbonate buffer has the potential to be a self sufficient and environmentally friendly technology for wastewater treatment if the CE is enhanced to recover sufficient amount of energy to offset the energy required for wastewater treatment. While the use of MFCs for wastewater treatment is in its infancy, MFCs as biosensors for environmental detection are nearing practical application [32,33]. Therefore, another benefit of an enhanced CE is to improve the application of MFCs for biochemical oxygen demand detection (BOD). An enhanced CE can improve the accuracy and reliability because more Coulombs pass through the circuit, which are proportional to the BOD values in the solution [34]. Another advantage of an enhanced CE is that it enhances MFC as batteries for power supply. MFCs have been used as power sources to drive low-power devices [35,36]; a higher CE can undoubtedly harvest more energy to sustain a longer battery life. As a result, this phosphorus-free anolyte has the potential to enhance the feasibility for practical applications of MFCs.

4. Conclusions

Compared with anolyte containing phosphate, this phosphorus-free anolyte effectively inhibits the growth and reproduction of microorganisms suspended in the solution and greatly reduces the suspended biomass in MFCs. The reduction of microorganisms suspended in the anolyte allows more substrate to be consumed by microorganisms attached on electrode surface for power generation. As a result, this phosphorus-free anolyte boosts the CE of MFCs. This study demonstrates that this phosphorus-free anolyte has the potential to enhance the feasibility for practical applications of MFCs.

Acknowledgments

This work was supported by a grant from the Environment & Water and Industry Development Council, Singapore (MEWR 651/06/159) and a grant from the Bill & Melinda Gates Foundation (OPP1069475). We also acknowledge the financial support from China Ocean Mineral Resources R&D Association (Grant No. DY125-15-T-08). Tang Xinhua thanks NUS Graduate School for Integrative Sciences and Engineering for a research scholarship support.

References

[1] K. Rabaey, W. Verstraete, Trends Biotechnol. 23 (2005) 291–298.
[2] B.E. Logan, Nat. Rev. Microbiol. 7 (2009) 375–381.
[3] H. Liu, R. Ramnarayanan, B.E. Logan, Environ. Sci. Technol. 38 (2004) 2281–2285.
[4] X.X. Cao, X. Huang, N. Boon, P. Liang, M.Z. Fan, Electrochem. Commun. 10 (2008) 1392–1395.
[5] P.L. McCarty, J. Bae, J. Kim, Environ. Sci. Technol. 45 (2011) 7100–7106.
[6] M. Di Lorenzo, T.P. Curtis, I.M. Head, K. Scott, Water Res. 43 (2009) 3145–3154.
[7] D.J. Wan, H.J. Liu, J.H. Qu, P.J. Lei, S.H. Mao, Y.N. Hou, Bioresour. Technol. 100 (2009) 142–148.
[8] M.H. Zhou, H.Y. Wang, D.J. Hassett, T.Y. Gu, J. Chem. Technol. Biotechnol. 88 (2013) 508–518.
[9] X.Y. Zhang, S.A. Cheng, P. Liang, X. Huang, B.E. Logan, Bioresour. Technol. 102 (2011) 372–375.
[10] B.E. Logan, B. Hamelers, R.A. Rozendal, U. Schrorder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Environ. Sci. Technol. 40 (2006) 5181–5192.
[11] B.R. Ringeisen, R. Ray, B. Little, J. Power Sources 165 (2007) 591–597.
[12] Z. He, N. Wagner, S.D. Minteer, L.T. Angenent, Environ. Sci. Technol. 40 (2006) 5212–5217.
[13] S.A. Cheng, D.F. Xing, B.E. Logan, Biosens. Bioelectron. 26 (2011) 1913–1917.
[14] J.J. Fornero, M. Rosenbaum, L.T. Angenent, Electroanalysis 22 (2010) 832–843.
[15] T.H.J.A. Sleutels, L. Darus, H.V.M. Hamelers, C.J.N. Buisman, Bioresour. Technol. 102 (2011) 11172–11176.
[16] J.C. Biffinger, J. Pietron, R. Ray, B. Little, B.R. Ringeisen, Biosens. Bioelectron. 22 (2007) 1672–1679.

[17] G. Reguera, K.P. Nevin, J.S. Nicoll, S.F. Covalla, T.L. Woodard, D.R. Lovley, Appl. Environ. Microbiol. 72 (2006) 7345–7348.
[18] K.Y. Cheng, G. Ho, R. Cord-Ruwisch, Environ. Sci. Technol. 42 (2008) 3828–3834.
[19] D.R. Bond, D.R. Lovley, Appl. Environ. Microbiol. 69 (2003) 1548–1555.
[20] G.C. Gil, I.S. Chang, B.H. Kim, M. Kim, J.K. Jang, H.S. Park, H.J. Kim, Biosens. Bioelectron. 18 (2003) 327–334.
[21] X.H. Tang, Z.W. Du, H.R. Li, Electrochem. Commun. 12 (2010) 1140–1143.
[22] L. Zhuang, Y. Yuan, G.Q. Yang, S.G. Zhou, Electrochem. Commun. 21 (2012) 69–72.
[23] H. Liu, S. Grot, B.E. Logan, Environ. Sci. Technol. 39 (2005) 4317–4320.
[24] S. Cheng, H. Liu, B.E. Logan, Environ. Sci. Technol. 40 (2006) 364–369.
[25] X.H. Tang, K. Guo, H.R. Li, Z.W. Du, J.L. Tian, Bioresour. Technol. 102 (2011) 3558–3560.
[26] D.R. Lovley, E.J.P. Phillips, Appl. Environ. Microbiol. 54 (1988) 1472–1480.
[27] S. Oh, B. Min, B.E. Logan, Environ. Sci. Technol. 38 (2004) 4900–4904.
[28] G.M. Walker, L.R. Weatherley, Environ. Pollut. 108 (2000) 219–223.
[29] O. Lefebvre, Z. Tan, S. Kharkwal, H.Y. Ng, Bioresour. Technol. 112 (2012) 336–340.
[30] S.R. Biswas, P. Ray, M.C. Johnson, B. Ray, Appl. Environ. Microbiol. 57 (1991) 1265–1267.
[31] P. Podsiadlo, S. Paternel, J.M. Rouillard, Z.F. Zhang, J. Lee, J.W. Lee, L. Gulari, N.A. Kotov, Langmuir 21 (2005) 11915–11921.
[32] H. Moon, I.S. Chang, J.K. Jang, K.S. Kim, J. Lee, R.W. Lovitt, B.H. Kim, J. Microbiol. Biotechnol. 15 (2005) 192–196.
[33] Y.F. Zhang, I. Angelidaki, Biotechnol. Bioeng. 108 (2011) 2339–2347.
[34] B.H. Kim, H.S. Park, H.J. Kim, G.T. Kim, I.S. Chang, J. Lee, N.T. Phung, Appl. Microbiol. Biotechnol. 63 (2004) 672–681.
[35] A. Dewan, C. Donovan, D. Heo, H. Beyenal, J. Power Sources 195 (2010) 90–96.
[36] H. Ren, H.S. Lee, J. Chae, Microfluid. Nanofluid. 13 (2012) 353–381.